IJPSR (2019), Volume 10, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 May 2018; received in revised form, 26 June 2018; accepted, 13 July 2018; published 01 January 2019

ESTIMATION OF QUERCETIN CONTENT IN THREE DIFFERENT SPECIES OF EUPATORIUM BY HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

V. Nithya and M. Kamalam *

Department of Botany, PSGR Krishnammal College for Women, Coimbatore - 641004, Tamil Nadu, India.

Keywords:

HPTLC, *Eupatorium glandulosum, Eupatorium odoratum, Eupatorium triplinerve*, quercetin

Correspondence to Author:
Dr. M. Kamalam

Associate Professor, Department of Botany, PSGR Krishnammal College for Women, Coimbatore - 641004, Tamil Nadu, India.

E-mail: kamaluma12@gmail.com

ABSTRACT: Three different species of *Eupatorium* namely *E. glandulosum, E. odoratum* and *E. triplinerve* belongs to the family Asteraceae are selected for the analysis of quercetin content. Leaves are extracted with ethanol and water and used for the analysis of quercetin by HPTLC technique using the mobile phase containing toluene: ethyl acetate: formic acid: methanol (5.5:4:1:0.5). Determination of quercetin content was performed by densitometric scanning under 254 nm, and the quercetin was detected at the R_f value of 0.54. The quantity of the quercetin content in plant extract was estimated by the calibration curve obtained from the standard quercetin. The result showed that the ethanolic extracts of *E. glandulosum* showed a high amount of quercetin (17.44 mg/g) followed by *E. odoratum* (13.4 mg/g) and *E. triplinrve* (9.29 mg/g).

INTRODUCTION: Plants produce a high diversity of secondary metabolites which are helpful in the defense mechanism of plants played against abiotic stress, and many of them have some medicinal importance. Quercetin is a flavonol, proved to be a potent antioxidant among poly-phenols [1, 2, 3]. It possesses antiviral, antibacterial, anticarcinogenic and anti-inflammatory effects [4, 5]. As per the literature, quercetin was reported in *E. glandulosum* [6], *E. perfoliatum* [7] and *E. cannabinum* [8]. In the present study, three different species of Eupatorium such as *E. glandulosum*, *E. odoratum* and *E. triplinerve* have been selected for the estimation of quercetin using High-Performance Thin Layer Chromatography.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(1).303-08

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(1).303-08

E. glandulosum belongs to the family Asteraceae is a native of Mexico, introduced as an ornamental shrub in several countries. In India, the tribes of Nilgiris use the leaves of this plant to heal wounds and small injuries [9]. *E. odoratum* is a fast growing perennial shrub, native of Central and South America has spread in tropical and subtropical regions of the world. The tribes of Indonesia used the leaf extract to cure skin diseases, poison bites, wounds, burns, cough, diabetes, diarrhea, fever, inflammation, and rheumatism. The boiled roots are used to cure urinary disorders [10, 11, 12].

E. triplinerve commonly called as Ayapana is an ornamental, erect, perennial herb having aromatic leaves. In tribal medicine, it is used to cure a fever with convulsions, pneumonia, indigestion, and cough [13]. Hence, to consider the medicinal importance of the above-said plants, the present study is undertaken with the objective of estimation of the quantity of quercetin content in three different species of *Eupatorium* using HPTLC technique.

MATERIALS AND METHODS:

Collection of Plant Material: The leaves of *E. glandulosum*, *E. odoratum*, and *E. triplinerve* were collected from Nilgiri Hills of Western Ghats, Coimbatore and Kanjikode Kerela respectively and certified by Botanical Survey of India, Coimbatore, Tamil Nadu. The plant materials are maintained in BSI Coimbatore under Voucher no: BSIR/RC/ 5/23/2017/Tech/338, BSIR/RC/5/23/2017/Tech/339 and BSIR/RC/5/23/2017/Tech/340. The leaves were washed thoroughly, dried, powdered and stored in air tight container for the study.

Preparation of Standard Solution: Quercetin (1mg/10ml) was prepared by dissolving 1 mg of quercetin in 10 ml of methanol in a standard flask.

Preparation of Plant Extracts: The leaf powder was defatted with petroleum ether and extracted with Ethanol (70 ºC) and water (100 ºC) in a Soxhlet apparatus. The extract was then dried and dissolved in a required amount of methanol.

Chromatography and Detection of Quercetin: Chromatography was performed on a 10 × 20 cm precoated HPTLC Silica gel 60 F_{254} plates (E-Merck, Mumbai, India). Aliquots of each of the extracts were separately applied (Samples and standard) to the plate as 6 mm wide band with an automatic TLC applicator Linomat-5 applicator (CAMAG, Switzerland), 5 mm from the bottom. The mobile phase consisted of Toluene: Ethyl acetate: Formic acid: methanol (5.5:4:1:0.5) was used per chromatography. The twin glass chamber was saturated with the mobile phase for about 30 minutes. The plate was developed up to 10 cm in twin glass horizontal developing chamber at the room temperature. Plates were air dried, and scanning was performed on a Camag TLC Scanner at 254 nm.

Calibration curve of Quercetin: The quercetin compound was determined by using a calibration curve established with a standard concentration ranging from 40 to 320 ng/spot. A stock solution of standard quercetin (l mg/ml) was prepared in methanol. The different volumes of stock solution 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8 and 3.2 ml were spotted on HPTLC plate to obtained concentration 40, 80, 120, 160, 200, 240, 280 and 320 ng/spot, respectively (bandwidth 6 mm, distance between tracks 7 mm) using automatic sample spotter. Peak areas were recorded for quercetin, and the calibration curve was obtained by plotting peak area against the concentration of quercetin.

RESULTS AND DISCUSSION: HPTLC fingerprinting of plant species is not only helps in the identification and quality control of a species but also to provide basic information useful for the isolation, purification, characterization, and identification of marker chemical compounds of the species. In the present study, methanol and water extracts of leaf powder of *E. glandulosum*, *E. odoratum* and *E. triplinerve* are used for analysis of quercetin content. Different concentrations of standard quercetin and leaf extracts were applied on HPTLC plates and developed in a solvent system consisting of toluene: ethyl acetate: formic acid: methanol (5.5: 4: 1: 0.5) and dried in air and scanned densitometrically **Fig. 1, 2a** and **2b**. The calibration curve of quercetin was found to be linear in the range of 4 to 32 mg/spot. A good linear relationship of standard quercetin was found to be R^2 = 0.998 concerning the concentration and peak area **Fig. 3** and **4**. The regression equation was found to be Y = 1268x + 1578 with respective to concentration.

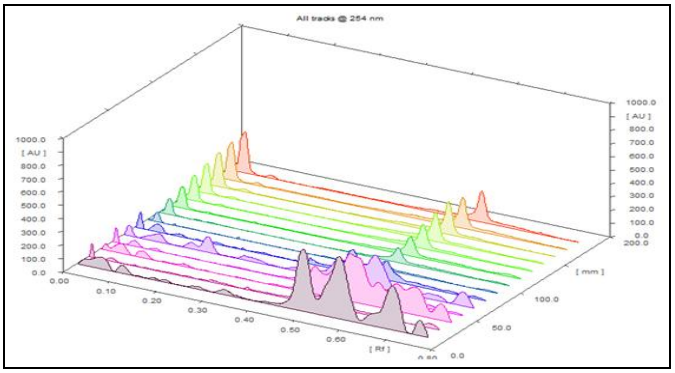

FIG. 1: DENSITOMETRIC CHROMATOGRAM OF QUERCETIN AND LEAF EXTRACTS (3D VIEW)

The total number of peaks was found to be 10, 12 and 12 in ethanol extracts of *E. glandulosum*, *E. odoratum*, *and E. triplinerve* respectively **Fig. 5**, **6** and **7**. The water extracts of *E. glandulosum*, *E. odoratum and E. triplinerve* showed 9, 7 and 10 peaks respectively **Fig. 8, 9** and **10**. The R_f value of standard quercetin was determined as 0.54 **Fig. 3**. In the plant samples, the ethanol and water extracts showed the peak at the R_f values of 0.54 and 0.55 **Fig. 5, 6, 7, 8, 9** and **10**.

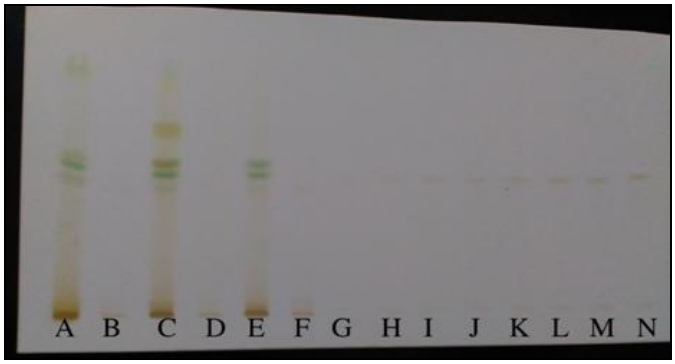

FIG. 2A: HPTLC PHOTOGRAPH OF SAMPLES AND VARIOUS CONCENTRATIONS OF QUERCETIN UNDER NORMAL LIGHT

FIG. 2B: HPTLC PHOTOGRAPH OF SAMPLES AND VARIOUS CONCENTRATIONS OF QUERCETIN UNDER UV LIGHT

A- Ethanol extract of *E. glandulosum*; B- Aqueous extract of *E. glandulosum*; C- Ethanol extract of *E. odoratum*
D- Aqueous extract of *E. odoratum*; E- Ethanol extract of *E. triplinerve*; F- Aqueous extract of *E. triplinerve*
G-N- Different concentration of quercetin

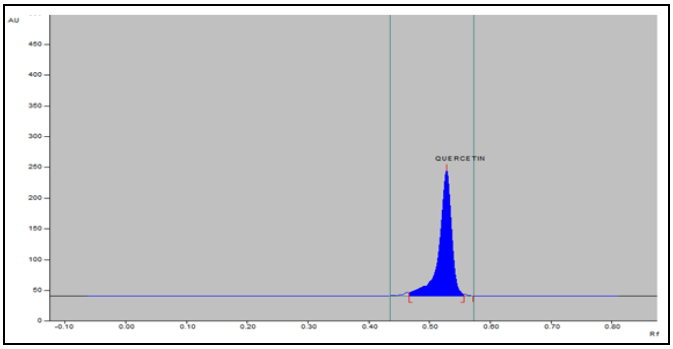

FIG. 3: HPTLC CHROMATOGRAM OF STANDARD QUERCETIN

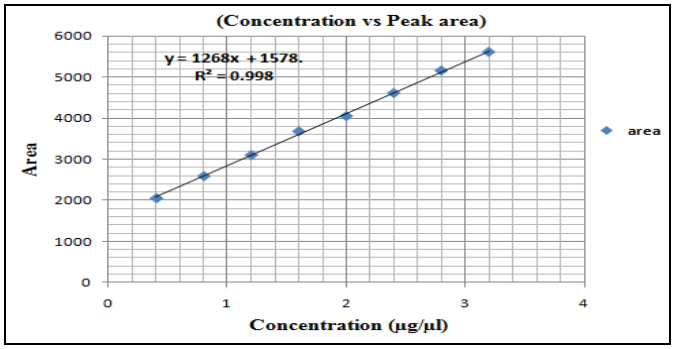

FIG. 4: CALIBRATION CURVE FOR STANDARD QUERCETIN

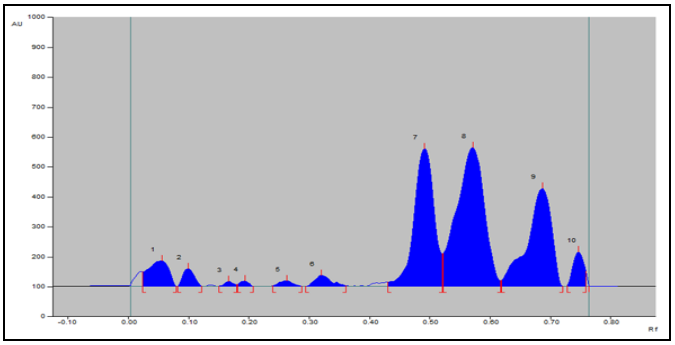

FIG. 5: HPTLC CHROMATOGRAM OF ETHANOL EXTRACT OF *EUPATORIUM GLANDULOSUM*

PEAK TABLE

Peak no.	R_f	Peak Area	Area (%)	Chemical substance
1	0.08	6176	12.85	Unknown
2	0.10	3091	5.90	Unknown
3	0.18	1346	2.52	Unknown
4	0.19	1529	2.79	Unknown
5	0.25	1348	2.52	Unknown
6	0.32	1861	2.89	Unknown
7	0.49	17016	28.50	Unknown
8	0.55	21395	37.74	Quercetin
9	0.69	13018	20.61	Unknown
10	0.75	6541	13.89	Unknown

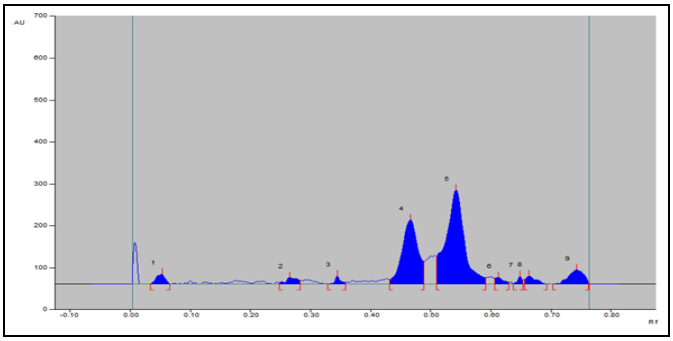

FIG. 6: HPTLC CHROMATOGRAM OF AQUEOUS EXTRACT OF *EUPATORIUM GLANDULOSUM*

PEAK TABLE

Peak no.	R_f	Peak Area	Area (%)	Chemical substance
1	0.05	2904	5.78	Unknown
2	0.26	492	0.85	Unknown
3	0.35	125	0.17	Unknown
4	0.47	5846	10.31	Unknown
5	0.54	6258	13.57	Quercetin
6	0.61	391	0.72	Unknown
7	0.64	101	0.10	Unknown
8	0.68	568	0.94	Unknown
9	0.75	3148	6.45	Unknown

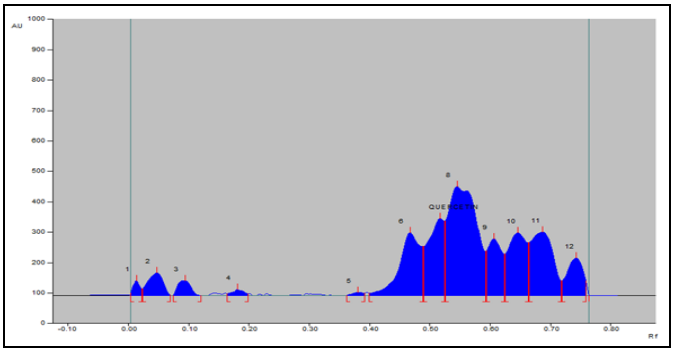

FIG. 7: HPTLC CHROMATOGRAM OF ETHANOLIC EXTRACT OF *EUPATORIUM ODORATUM*

PEAK TABLE

Peak no.	R_f	Peak Area	Area (%)	Chemical substance
1	0.01	381	0.70	Unknown
2	0.05	546	0.84	Unknown
3	0.08	421	0.74	Unknown
4	0.19	184	0.32	Unknown
5	0.39	116	0.13	Quercetin
6	0.48	9724	17.52	Unknown
7	0.52	10485	19.21	Unknown
8	0.55	14157	23.42	Quercetin
9	0.62	8485	15.96	Unknown
10	0.65	9834	17.64	Unknown
11	0.69	9842	17.91	Unknown
12	0.73	3461	6.85	Unknown

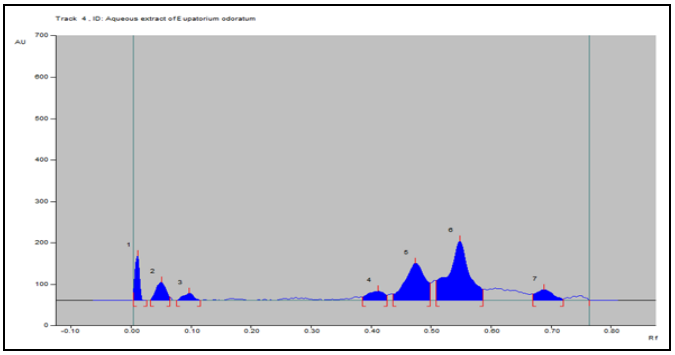

FIG. 8: HPTLC CHROMATOGRAM OF AQUEOUS EXTRACT OF *EUPATORIUM ODORATUM*

PEAK TABLE

Peak no.	R_f	Peak Area	Area (%)	Chemical substance
1	0.01	495	0.86	Unknown
2	0.05	542	0.92	Unknown
3	0.09	263	0.41	Unknown
4	0.42	932	1.40	Unknown
5	0.48	2663	5.41	Unknow
6	0.55	4411	7.85	Quercetin
7	0.69	945	1.53	Unknown

Among the selected three plants, the ethanol extract of *E. glandulosum* showed the maximum amount of quercetin (17.44 mg/g) followed by *E. odoratum* (13.40 mg/g) and *Eupatorium triplinerve* (9.29 mg/g). Water extracts showed a minimum amount of quercetin (2.67 mg/g) in *E. triplinerve* **Table 1** and **2**.

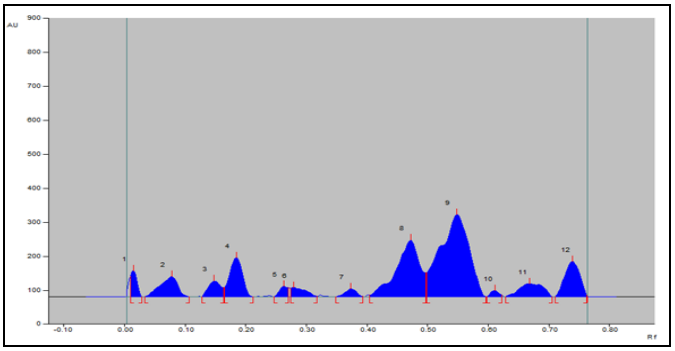

FIG. 9: HPTLC CHROMATOGRAM OF ETHANOLIC EXTRACT OF *EUPATORIUM TRIPLINERVE*

PEAK TABLE

Peak no.	R_f	Peak Area	Area (%)	Chemical substance
1	0.01	452	0.77	Unknown
2	0.08	2016	5.02	Unknown
3	0.15	942	1.52	Unknown
4	0.19	5249	10.16	Unknown
5	0.27	352	0.64	Unknown
6	0.28	395	0.75	Unknown
7	0.38	241	0.37	Unknown
8	0.48	8321	15.36	Unknown
9	0.55	10655	19.42	Quercetin
10	0.61	132	0.24	Unknown
11	0.66	236	0.36	Unknown
12	0.73	5245	10.15	Unknown

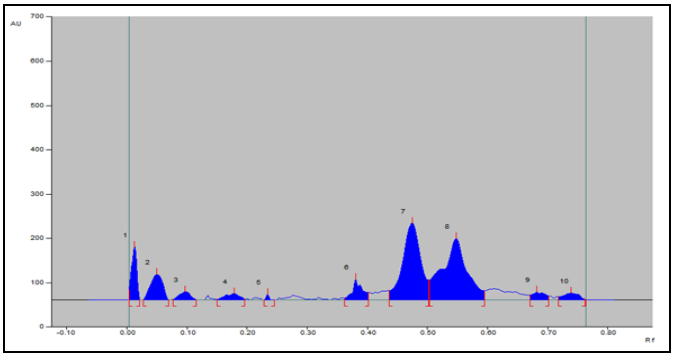

FIG. 10: HPTLC CHROMATOGRAM OF AQUEOUS EXTRACT OF *EUPATORIUM TRIPLINERVE*

PEAK TABLE

Peak no.	R_f	Peak Area	Area (%)	Chemical substance
1	0.01	2165	5.18	Unknown
2	0.05	2542	5.39	Unknown
3	0.10	598	0.96	Unknown
4	0.18	456	0.79	Unknown
5	0.23	57	0.02	Unknown
6	0.38	1064	2.37	Unknown
7	0.48	5946	10.73	Unknown
8	0.55	5013	10.02	Quercetin
9	0.69	325	0.64	Unknown
10	0.73	418	0.73	Unknown

Apart from quercetin, all the leaf extracts showed many numbers of peaks at different R_f values which shows the presence of various other compounds.

Similarly, the ethanolic extract of *Calamus rotang* showed the R_f value of quercetin at 0.54 [14,] but in contrast, a polyherbal syrub zymodyne and methanolic leaf and flower extract of *Moringa oleifera* showed the presence of quercetin at the R_f value of 0.86 and 0.35 respectively [15, 16]. The HPTLC analysis of an aqueous extract of *Eruca sativa* was found to be 17.94 [17] which is equal to *E. glandulosum*.

TABLE 1: PEAK TABLE OF QUERCETIN IN LEAF EXTRACTS

S. no.	Plant name	Solvent name	Total number of peaks	Peak no.	R_f	Peak area	Area (%)
1	*Eupatorium glandulosum*	Ethanol	10	8	0.55	21395	37.74
2	*Eupatorium glandulosum*	Aqueous	9	5	0.54	6258	13.57
3	*Eupatorium odoratum*	Ethanol	12	8	0.55	14157	23.42
4	*Eupatorium odoratum*	Aqueous	7	6	0.55	4411	7.85
5	*Eupatorium triplinerve*	Ethanol	12	9	0.55	10655	19.42
6	*Eupatorium triplinerve*	Aqueous	10	8	0.55	5013	10.02

As per the literature, quercetin possesses biological and therapeutic effects including anti-cancer, anti-oxidative, anti-microbial and anti-inflammatory, cardioprotective and hepatoprotective activities [18, 19, 20]. Hence, from the above findings, it is confirmed that the leaf possesses a good amount of quercetin content.

TABLE 2: QUERCETIN CONTENT IN PLANT EXTRACTS

S. no.	Name of the plant extract	Amount of Quercetin (mg/g)	
		Ethanol extract	Water extract
1	*E. glandulosum*	17.44	2.92
2	*E. odoratum*	13.40	3.74
3	*E. triplinerve*	9.29	2.67

CONCLUSION: The quantitative estimation of quercetin was analyzed in three different species of *Eupatorium* leaves by HPTLC fingerprinting technique. The quercetin content was found to be maximum in the ethanolic extract of all the three plants than water extract. The ethanolic extract of *E. glandulosum* leaves showed maximum quercetin content than other two species studied. Since, the leaves possess the promising amount of quercetin, it can be used for curing various ailments.

ACKNOWLEDGEMENT: We are grateful to thank the Principal, PSG College of Pharmacy for providing facilities to complete this work.

CONFLICT OF INTEREST: The authors declare no conflicts of interest.

REFERENCES:

1. Panya T, Chansri N, Sripanidkulchai B and Daodee S: Additional antioxidants on the determination of quercetin from *Moringa oleifera* leaves and variation content from different sources. International Food Research Journal 2018; 25(1): 51-55.
2. Lesjak M, Beara I, Simin N, Pintać D, Majkic T, Bekvalac K, Orcic D and Mimica-Dukic N: Anti-oxidant and anti-inflammatory activities of quercetin and its derivatives. Journal of Functional Foods 2018; 40: 68-75.
3. Zheng YZ, Deng G, Liang Q, Da-Fu C, Guo R and Rong-Cai L: Anti-oxidant activity of quercetin and its glucosides from propolis: a theoretical study. Scientific Reports 2017; 7: 1-11.
4. Wu W, Li R, Li X, He J, Jiang S, Liu S and Yang J: Quercetin as an antiviral agent inhibits Influenza A Virus (IAV) Entry. Viruses 2016; 8(1): 1-18.
5. Berakdar N, Rawaa AL, Kayali and Sabbagh G: *In-vitro* antibacterial activity of genistein and quercetin against *Escherichia coli* isolated from clinical samples. Innovare Journal of Life sciences 2016; 4(4): 5-8.
6. Mukherjee PK, Mukherjee K, Hermans-Lokkerbo ACJ, Verpoorte R and Suresh B: Flavonoid content of *Eupatorium glandulosum* and *Coolebroke oppositifolia*. Journal of natural remedies 2001; 1(1): 21-24.
7. Maas M, Petereit F and Hensel A: Caffeic acid derivatives from *Eupatorium perfoliatum* L. Molecules 2009; 14(1): 36-45.
8. Ionita L, Grigore A, Pirvu L, Draghici E, Corina C, Ionita C, Panteli M and Dobre N: Pharmacological activity of an *Eupatorium cannabinum* L. extract 2013; 18(6): 8779-8786.
9. Desingh M, Jesudas JM, Balasubramaniam P, Karthikeyan, Mayakrishnan, Ganesan B and Mohan R: Phytochemical analysis and anti-microbial activity of *Eupatorium glandulosum*. International Journal of Current Microbiology and Applied Science 2014; 3(7): 882-885.
10. Taylor RSL, Hudson JB, Manandhar NP and Towers GHN: Antiviral activities of medicinal plants of southern Nepal. J., Ethanopharmacol 1996; 53: 97-102.
11. Irobi ON: Antibiotic properties of ethanol extract of *Chromolaena odorata* (Asteriaceae). Inter J Pharmacognosy 1997; 35: 111-126.
12. Amatya S and Tuladhar SM: *In-vitro* antioxidant activity of extracts from *Eupatorium odoratum*. Res J L Medicinal Plant 2011; 5: 79-86.
13. Hossan S, Hanif A, Khan M, Bari S, Jahan R and Rahmatullah M: Ethnobotanical survey of the Tripura tribe of Bangladesh. American-Eurasian Journal of Sus tainable Agriculture 2009; 3(2): 253-261.
14. Pallavi Y and Hemalatha KPJ: Phytochemical studies and High-Performance Thin Layer Chromatography analysis of *Calamus rotang* linn leaf extracts. Asian Journal of Pharmaceutical & Clinical Research 2018; 11(2): 269-275.

15. Parmar S, Shah N, Kasarwala M, Virpura M, Shah K and Patel P: Determinations of quercetin by HPTLC method present in zymodyne syrup- a poly herbal formulation. International J Pharmaceutical Sciences and Research 2011; 2(10): 2724-2728.
16. Meghani N, Jethra B, Shah R and Nagendran S: Fingerprint analysis for validation and simultaneous quantification of quercetin and kaempferol in *Moringa oleifera* by RP-HPLC and HPTLC. International J Pharmaceutical Sciences and Research 2018; 9(4): 1499-1510.
17. Sajeeth CI, Manna PKR and Manavalan Jolly CI: Quantitative estimation of Gallic Acid, Rutin and Quercetin in certain herbal plants by HPTLC method. Pelagia Research Library 2010; 1(2): 80-85.
18. Harwood M, Danielewska-Nikiel B, Borzelleca JF, Flamm GW, Williams and GM and Lines TC: A critical review of the data related to the safety of quercetin and lack of evidence of *in-vivo* toxicity, including lack of genotoxic/ carcinogenic properties. Food Chem Toxicol 2007; 45: 2179-2205.
19. Hernandez-Ortega LD, Alcantar-Dıaz BE, Ruiz-Corro LA, Sandoval-Rodriguez A, Bueno-Topete M, Armendariz-Borunda J and Montes SAM: Quercetin improves hepatic fibrosis reducing hepatic stellate cells and regulating pro-fibrogenic / anti-fibrogenic molecules balance. J Gastroenterol Hepatol 2012; 27: 1865-1872.
20. Andrea G: Quercetin: a flavonol with multifaceted therapeutic applications. Fitoterapia 2015; 106: 256-271.

How to cite this article:
Nithya V and Kamalam M: Estimation of quercetin content in three different species of *Eupatorium* by high performance thin layer chromatography. Int J Pharm Sci & Res 2019; 10(1): 303-08. doi: 10.13040/IJPSR.0975-8232.10(1).303-08.